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Personal exposures to $PM_{2.5}$ and polycyclic aromatic hydrocarbons and their relationship to environmental tobacco smoke at two locations in Greece

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In the context of a large-scale molecular epidemiology study of biomarkers of genotoxicity of air pollution, 24-h mean personal exposures to airborne $PM_{2.5}$ (particulate matter <2.5 μm) and associated polycyclic aromatic hydrocarbon (PAHs) were measured in 194 non-smoking technical institute students living in the city of Athens, Greece (an area with moderately high levels of air pollution) and the nearby small town of Halkida anticipated to have lower pollution levels. Extensive information relevant to the assessment of long-term and recent exposure to PAH was obtained from questionnaires as well as a time–location–activity diary (TLAD) which was kept by all subjects during a 4-day observation period. During the last 24 h of this period, subjects underwent personal exposure monitoring for $PM_{2.5}$ and PAH, while a sample of blood was donated at the end of this period. All subjects were monitored in this way twice; once during a winter season (October–February) and once during the following summer season (June–September). Nine subjects with plasma cotinine levels above 20 ng/ml were considered as unreported smokers and excluded from the study. Winter $PM_{2.5}$ exposures were lower in Athens (geometric mean 39.7 $\mu g/m^3$) than Halkida (geometric mean 56.2 $\mu g/m^3$) ($P<0.001$), while there was no significant location difference during the summer (Athens: geometric mean 32.3 $\mu g/m^3$, Halkida: geometric mean 32.9 $\mu g/m^3$; $P\approx 0.79$). On the other hand, PAH exposures (sum of the eight carcinogenic PAHs) were significantly higher in Athens than in Halkida during the winter (Athens: geometric mean 8.26 ng/m^3, Halkida: geometric mean 5.80 ng/m^3; $P<0.001$) as well as during the summer (Athens: geometric mean 4.44 ng/m^3, Halkida: geometric mean 1.48 ng/m^3; $P<0.001$). There was a significant difference in the profile of the PAH exposures at the two locations, the proportion of lighter PAH (benzo[*a*]anthracene, chrysene [CHRYS], benzo[*k*]fluoranthene, and benzo[*b*]fluoranthene) being higher, and that of heavier PAH (benzo[*ghi*]perylene [BPer] and indeno[1,2,3,*cd*]pyrene) lower, in Halkida than in Athens, regardless of season. This difference appeared to be related to individual exposure to environmental tobacco smoke (ETS), as indicated by (a) the correlation at the individual level between the CHRYS/BPer ratio and declared time of recent exposure to ETS as well as plasma cotinine levels, especially during the winter; (b) the parallel variation of the mean levels of all three markers (declared ETS exposure, cotinine levels, CHRYS/BPer ratio) among three subgroups of subjects (Athens subjects who had lowest levels of all three markers; Halkida subjects other than those living in the institute campus area; and Halkida subjects living in the institute campus area who had the highest levels of all three markers). This demonstrates that ETS can have a distinctive effect on the PAH exposure profile of subjects exposed to relatively low levels of urban air pollution. *Journal of Exposure Analysis and Environmental Epidemiology* (2001) **11**, 169–183.

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Introduction

Urban air pollution originates mainly from incomplete fossil fuel combustion. Sources of emission of $PM_{2.5}$ (particulate matter <2.5 μm) and their associated polycyclic aromatic hydrocarbon (PAH) within the urban environment are predominantly associated with traffic, domestic heating, power generation and other industrial activities (Nikolaou et al., 1984; Back et al., 1991). In addition to PAH emission sources located within urban centres, point sources (e.g., industrial plants) located at long distances from such centres have also been reported to affect urban air quality (Lunde and Bjorseth, 1977; Bjorseth and Lunde, 1979). The composition of the released gaseous or particulate-

1. Abbreviations: PAH, polycyclic aromatic hydrocarbon; $PM_{2.5}$, particulate matter <2.5 μm; ETS, environmental tobacco smoke; B[*a*]A, benzo[*a*]anthracene; CHRYS, chrysene; B[*b*]F, benzo[*b*]fluoranthene; B[*k*]F, benzo[*k*]fluoranthene; B[*a*]P, benzo[*a*]pyrene; DBA, benzo[*a*,*h*]anthracene; BPer, benzo[*ghi*]perylene; IND, indeno[1,2,3,*cd*]-pyrene; TLAD, time–location–activity diary; TEI, Technical Educational Institute.

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bound mixtures is complex, including more than 600 chemicals. Among these chemicals, PAHs, especially the high-molecular-weight ones (four to seven rings), are of particular interest because many of them can be metabolically activated *in vivo* to DNA-reactive intermediates and are known to be mutagenic and carcinogenic in rodents (IARC Monographs, 1984). Because of their low volatility, these heavy PAHs are predominantly found associated with the respirable fraction of suspended particulate matter ($PM_{2.5}$) (Pott, 1983; Shen et al., 1997).

The International Agency for Research in Cancer (IARC) has classified exhaust from diesel-powered engines as a probable human carcinogen (category 2A) and exhaust from gasoline-powered engines as a possible human carcinogen (category 2B) (IARC Monographs, 1989). Although epidemiological studies have indicated an increased risk of lung cancer for city dwellers (Katsouyanni and Pershagen, 1997), the extent to which urban air PAH may contribute to human cancer risk is not known with certainty. An increased risk for lung and bladder cancer has been documented for cohorts occupationally exposed to high levels of PAH mixtures (i.e., coke oven, iron foundry, steel, and tar workers) (Muscat and Wynder, 1995). On the other hand, studies on professional groups such as bus and truck drivers and railroad workers, who, as a result of their outdoor activities, suffer extended exposure to ambient urban (rather than workplace) air pollution, have given inconclusive or contradictory results (reviewed in Mastrangelo et al., 1996).

Over the past decade, human biomarker studies have attempted to assess the genotoxic consequences of exposure to air pollution at the individual level, examine individual susceptibility to airborne genotoxins, and reveal early biological effects that might have a negative impact on human health. Such studies have demonstrated in many cases increased risk for genetic damage (increased levels of DNA adducts and chromosomal damage) in populations occupationally exposed to high levels of PAH mixtures arising from industrial point sources or present in the general urban atmosphere (Anwar and Kamal, 1988; Perrera et al., 1992, 1993; Santella et al., 1993; Binkova et al., 1995, 1998; Chandrasekaran et al., 1996). The levels of air pollution, which were found in such studies to give rise to detectable genotoxic damage in exposed populations, lie within ranges generally considered beyond those normally found in most Western European or US cities, where, in studies conducted since 1980, typical average benzo[*a*]pyrene (B[*a*]P) levels have generally been reported to lie in the range of about 1–5 ng/m^3 in Europe and about 1 ng/m^3 in the US (Menichini, 1992). On the other hand, analogous studies on populations exposed to moderate to low levels of urban air PAH are limited and have not yielded clear results (for reviews, see Georgiadis and Kyrtopoulos, 1999; Sram and Binkova, 2000). Many of the studies, which have looked for chronic effects of moderate to low urban air pollution, have based their estimation of exposure on measurements of the ambient concentrations of "classic" air pollutants (mainly SO_2, CO, NO_x, and ozone) or, more rarely, of ambient concentrations of PAH measures using fixed site monitoring stations.

Ambient PAH exposure was measured in numerous studies for urban, industrial, or rural environments (reviewed by Pott, 1983; Menichini, 1992), and the PAH profiles from different mobile or stationary sources were reported (IARC Monographs, 1983; reviewed by Daisey et al., 1986). Other studies were focused on the quantitative and qualitative differences of indoor and outdoor PAH exposure and the possible effects of indoor activities (e.g., cooking) on the air quality within the house premises (Chuang et al., 1991; Koo et al., 1994; Zheng et al., 1997; Dubowsky et al., 1999). The results of these studies indicate that under conditions of relatively low levels of ambient air pollution, variations in factors such as personal lifestyle and outdoor activities may significantly modify individual PAH exposure, thus limiting the usefulness of ambient (rather than personal) exposure measurements. For this reason, in biomarker population studies, measurement of personal exposures is needed to provide a more accurate measurement of human exposure at the microenvironment level (Mage and Buckley, 1995).

The AULIS project, within which the currently reported work was carried out, is a large-scale molecular epidemiological study conducted in the context of the European Union Environment and Climate Programme to investigate, in cohorts of the general population suffering from moderate to low levels of exposure to urban air pollution, the quantitative relationships between $PM_{2.5}$ and PAH exposure, on one hand, and the levels of various biomarkers related to exposure to, early effects of, and susceptibility to, genotoxins on the other.

The cohorts studied included subjects living at two locations in Greece, the city of Athens, and the neighbouring provincial town of Halkida. Athens was chosen because it is a city characterised by (a) dense urban agglomeration (about 4 million inhabitants); (b) heavy traffic burden (more than 1.5 million vehicles, 1.1 million of which are gasoline-driven private cars while the rest are diesel-powered trucks, taxis, and buses); (c) high temperatures and high levels of solar radiation throughout the year leading to intense photochemical activity (very high ozone concentrations are frequently observed); and (d) poor ventilation due to its location in a basin surrounded by mountains. For these reasons, the city suffered until recent years from serious problems of air pollution, with the levels of primary or secondary air pollutants often exceeding the air quality standards of the European Union. Traffic constitutes the major source of air pollution in Athens, especially during the warmer part of the year, while during

winter (November to March), diesel-based central heating (the most common means of home heating in the city) is an additional significant source of pollution. Although there is significant industrial activity around Athens, this is mainly of small to medium scale and its contribution to the city's air pollution is thought to be of secondary importance. The poor state of the atmospheric environment in Athens in earlier years was reflected in the levels of standard indices such as ambient concentrations of black smoke, NO, CO, and SO_2 (black smoke is an indicator of ambient particles measured routinely in Athens during the study period and until very recently; it represents concentrations of black particles with an aerodynamic diameter <4.5 μm and has a long history in Europe) (Department of Health, Committee on the Medical Effects of Air Pollution, 1995). For example, mean annual levels at a monitoring station in the centre of Athens in 1988 were reported to be 147 $\mu g/m^3$ for black smoke, 182 $\mu g/m^3$ for NO, 7.4 mg/m^3 for CO, and 82 $\mu g/m^3$ for SO_2) (Hellenic Ministry for the Environment, 1999). Control measures taken during the past decade, such as use of better diesel quality for residential heating and substitution of older technology cars with cars with catalytic converters, have resulted in improvements in most indices of air quality. Thus, by 1998 at the same monitoring station mentioned above (which always shows, by far, the highest levels of pollutants among the various monitoring stations of Athens), mean ambient levels had dropped to 117 $\mu g/m^3$ for black smoke, 129 $\mu g/m^3$ for NO, 5.6 $\mu g/m^3$ for CO, and 37 $\mu g/m^3$ for SO_2 (Hellenic Ministry for the Environment, 1999). These levels for SO_2, NO, and CO are comparable to those found in many cities of Western Europe of similar size and population density, and are far lower than the levels reported for many Eastern European cities. However, black smoke levels continue to be among the highest observed in Western Europe and are within the range considered to be dangerous for human health.

In view of the high personal exposures to $PM_{2.5}$ and PAH anticipated to be found for the Athens cohort in the AULIS study, and in an effort to extend the range of exposures to be studied to lower levels, an additional cohort was included in the study consisting of subjects living in the area of Halkida, a town of 25,000 inhabitants, 70 km north of Athens, having a low traffic density and little industrial activity in its vicinity. No information on ambient pollution levels at Halkida was available at the time of initiation of the project, but consideration of the town's demographic and economic characteristics suggested that they would probably be lower than those of Athens.

The study cohorts consisted of non-smoking subjects who, in the context of the study, had their personal exposure to airborne $PM_{2.5}$ and associated PAH measured, as well as a series of biomarkers relevant to the assessment of PAH genotoxicity, including urinary PAH metabolites, plasma cotinine, bulky DNA adducts, HPRT mutations, chromosome aberrations, and sister chromatid exchanges in blood lymphocytes. The effects of polymorphisms in a series of carcinogen-metabolising genes on biomarker levels were also examined. Here we present results on individual exposures to $PM_{2.5}$ and PAH, and their relationships to some exposure-modifying parameters. In subsequent publications, the relationship of these exposures to various exposure and effect biomarkers will be discussed.

Methods

Population Study

Details of the design and practical conduct of the field study have been reported elsewhere (Kyrtopoulos and Georgiadis, submitted). Briefly, the population study was conducted in four phases: two winter periods — running from November 1996 to February 1997 and from November 1997 to February 1998 — and two summer periods — running from May 1997 to September 1997 — and during a similar period in 1998. Two hundred three non-smoking Technical Educational Institute (TEI) full-time students, all volunteers, 19–24 years old, were enrolled into the study. Each subject was monitored twice, once during the winter and again during the following summer period. In an attempt to exclude unreported smokers, cotinine levels were measured in plasma samples. Subjects had been informed prior to the field study that their smoking status would be checked biochemically. Nine individuals failed to pass the plasma cotinine level criteria (see below) and thus, the study was based on 194 subjects (including 58 males and 136 females). One hundred seventeen subjects lived in Athens and attended Athens TEI, while 77 lived in Halkida or its surroundings and attended Halkida TEI. Subjects were monitored in groups of five, two groups per week, with weekly alternation between the two locations. All participants answered a personal history questionnaire giving information on their place of residence, dietary habits, health history, and activities that might influence their exposure to agents of interest for the study. In addition, during a 4-day observation period, they kept a detailed time–location–activity dairy (TLAD) where they indicated information on their location and activities every 15 min. During the last 24 h of this observation period, they carried a personal monitor for $PM_{2.5}$. At the end of this period, subjects provided a sample of blood and answered a further questionnaire giving additional information on their activities during the previous 24 h. All subjects had the purpose of the study explained to them and signed an informed consent form.

Personal Exposure Monitoring

For monitoring of external exposure, respirable particulates ($PM_{2.5}$) were collected on Gelman Teflon filters using

small $PM_{2.5}$ GK2.05 cyclones, which have been designed and constructed for the EXPOLIS study by BGI (Waltham, MA, USA) (Kenny and Gussman, 1997; Jantunen et al., 1998). These personal samplers are equipped with a 2.5-μm particle size cut-off point attached to a battery-operated pump (Buck IH, Orlando, Florida). The samplers were operated at 4 l/min. Flow rates were adjusted just prior to monitoring and measured again at the end of the 24-h period. Before use and following the end of sample collection, filters were weighted at the balance (Perkin Elmer A4) of the Institute of Geological Research, which has a resolution of 1 μg. Every time filters were weighted, the atmospheric pressure, the temperature, and the humidity in the room were recorded before and after weighing. Humidity ranged between 26% and 66% and temperature between 20°C and 25°C. In addition, a standard weight and three laboratory blanks were weighed. Before weighing, the filters were discharged with a static charge eliminator (Multistat). Every filter was weighed twice. If the difference in weight between the two times was less than or equal to 3 μg, this measurement was considered acceptable and the final weight was taken as the average of the two. If the difference was greater than 3 μg, the procedure was repeated. Filters were placed in Petri dishes and stored at −20°C in the dark until PAH analysis.

Independent tests showed that there was no loss of PAH during the storage period of the filters.

PAH Analysis

PAH analysis was performed at the National Hellenic Research Foundation using a modification of US EPA method 610 (US EPA, 1984). PAHs were quantified by the use of external standards (Hewlett-Packard) as well as an internal standard (10-fluoro-biphenal). Filters were subjected to overnight (16 h) extraction with dichloromethane (350 ml) in a Soxhlet apparatus in the presence of the internal standard, and extracts were concentrated to 5 ml in Kuderna–Danish concentrators. After a solvent exchange step to acetonitrile, crude extracts were concentrated to less than 0.5 ml, and the final volume was adjusted to 1 ml. Twenty microliters of each sample was analysed by HPLC using a PAH-specific column (Lichrosphere C-18-PAH 3 mm column; gradient 60/40 acetonitrile/water to 100% acetonitrile) using a time-programmable fluorescence detector to ensure maximum sensitivity and selectivity. Eight carcinogenic PAHs were quantified: benzo[*a*]anthracene (B[*a*]A), excitation 287 nm, emission 386 nm; chrysene (CHRYS), excitation 265 nm, emission 380 nm; benzo[*b*]fluoranthene (B[*b*]F), excitation 290 nm, emission 430 nm; benzo[*k*]fluoranthene (B[*k*]F), excitation 290 nm, emission 430 nm; B[*a*]P, excitation 290 nm, emission 430 nm; benzo[*a,h*]anthracene (DBA), excitation 290 nm, emission 410 nm; benzo[*ghi*]perylene (BPer), excitation 290 nm, emission 410 nm; indeno[1,2,3,*cd*]pyrene (IND), excitation 300 nm, emission 500 nm. The sum of the eight carcinogenic PAH will be referred to as total PAH.

Cotinine Measurements

Plasma cotinine levels were analysed at the Institute of Experimental Medicine Acad. Sci. C.R. and Regional Institute of Hygiene of Central Bohemia in Prague by radioimmunoassay (Van Vunakis et al., 1987) using the RIA set provided by Brandeis University (Waltham, MA, USA). Plasma was isolated from heparinised blood by 10-min centrifugation at 1000×*g*. Seven cotinine standards (Sigma) in the range of 0.05–5 ng/ml were used in duplicates to construct the standard curve in log–logit scale. When the plasma levels of cotinine were higher than 5 ng/ml (highest concentration of standard), samples were diluted accordingly with reaction buffer. The detection limit corresponds to the lowest standard value of 0.05 ng/ml. The variability between experiments was checked by using three plasma samples with known levels of cotinine: low (1 ng/ml), average (10 ng/ml), and high (100 ng/ml). All samples were analysed in duplicate. If the difference between duplicates was higher than 15%, the analysis was repeated.

Statistical Analysis

All measured values were ln-transformed, normal distributions were obtained, and Student's independent samples *t*-test, one-way ANOVA, and linear regression analysis were used. Pearson correlation analysis was used to examine the association between individual PAH and $PM_{2.5}$ values. The SPSS v 8.0 software was used.

Results

Eleven samples were found to have cotinine levels above 20 ng/ml (Figure 1), and the corresponding nine individuals (two individuals had cotinine levels above 20 ng/ml during both sampling seasons) were considered as possible active smokers and for this reason, all data from them were excluded from the study.

Personal Exposure to $PM_{2.5}$ and PAH

The winter geometric mean $PM_{2.5}$ personal exposure values were 39.7 $\mu g/m^3$ for Athens subjects and 56.2 $\mu g/m^3$ for Halkida subjects (Table 1) (an outlier with a $PM_{2.5}$ exposure higher than 600 $\mu g/m^3$ was omitted for the rest of the study), indicating higher particle exposure in Halkida than in Athens ($P<0.001$) (Figure 2A). On the other hand, no significant difference between the two locations was observed during the summer collection period, geometric mean $PM_{2.5}$ exposure being 32.3 $\mu g/m^3$ in Athens and 32.9 $\mu g/m^3$ in Halkida ($P=0.79$). A seasonal variation was

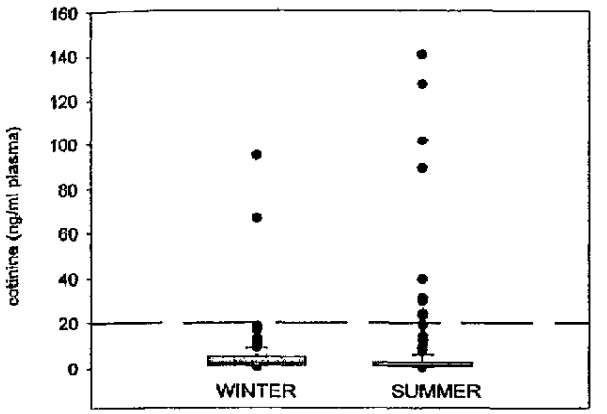

Figure 1. Plasma cotinine levels in winter and summer samples. Individuals with high cotinine levels (> 20 ng/ml; dashed line) during either winter or summer collection period were considered as possible active smokers and excluded from the study.

clearly evident, the geometric mean $PM_{2.5}$ exposures being significantly higher during the winter than during the summer period in both Athens (P=0.015) and Halkida (P<0.001).

Figure 2B shows the total PAH exposures (based on the sum of the eight carcinogenic PAHs) at both locations and during both seasons. In contrast to what was observed with $PM_{2.5}$ during winter, Athens subjects were exposed to higher levels of PAH than Halkida subjects, geometric mean exposure levels being 8.26 $\mu g/m^3$ for Athens and 5.80 $\mu g/m^3$ for Halkida (P<0.01) (Table 1). The exposure difference between the two locations was even more pronounced during the summer period, with geometric mean exposure levels in Athens being 4.44 $\mu g/m^3$ and in Halkida 1.48 $\mu g/m^3$ (P<0.001). Comparing exposures during the different monitoring seasons, it can be seen that the summer PAH exposure (geometric mean) was reduced 1.9-fold compared to winter in Athens (P<0.001), while the summer reduction in Halkida was much greater (3.9-fold; P<0.001).

High inter-individual variation was observed in both $PM_{2.5}$ and PAH exposures, even among individuals sampled on the same day (data not shown). It is worth mentioning that individuals living in the centre of Athens (Municipality of Athens), which is the most polluted region of the Greater Athens Area, were exposed to higher PAH levels (pooled winter and summer samples) relative to all the other Athenian subjects (geometric means: PAH 9.27 and 7.14 ng/m^3, respectively; P=0.059). In a multiple linear regression model, $PM_{2.5}$ during the winter period correlated with both the place of residence and declared time of environmental tobacco smoke (ETS) exposure during the last 24 h at P<0.05, while total PAH correlated at P<0.05 level only with the declared time of ETS (Table 2). In summer, none of the potential correlations was significant at

Table 1. Personal $PM_{2.5}$ and PAH exposures at both locations and both seasons; P values were obtained from Students' t-test of the ln-transformed values.

	Mean	Median	Geometric mean	Range	P
$PM_{2.5}$ ($\mu g/m^3$)					
Winter, Athens	46.4	40.5	39.7	7.5–140.4	<0.001
Winter, Halkida	66.0	58.9	56.2	9.8–259.8	
Summer, Athens	35.8	32.5	32.3	4.9–125.0	0.79
Summer, Halkida	37.9	31.6	32.9	10.6–233.4	
PAH (sum of eight carcinogenic PAHs; ng/m^3)					
Winter, Athens	10.87	7.81	8.26	1.23–50.24	<0.01
Winter, Halkida	7.25	6.24	5.80	0.81–29.31	
Summer, Athens	5.03	4.47	4.44	1.29–13.9	<0.001
Summer, Halkida	1.81	1.62	1.48	0.27–8.60	

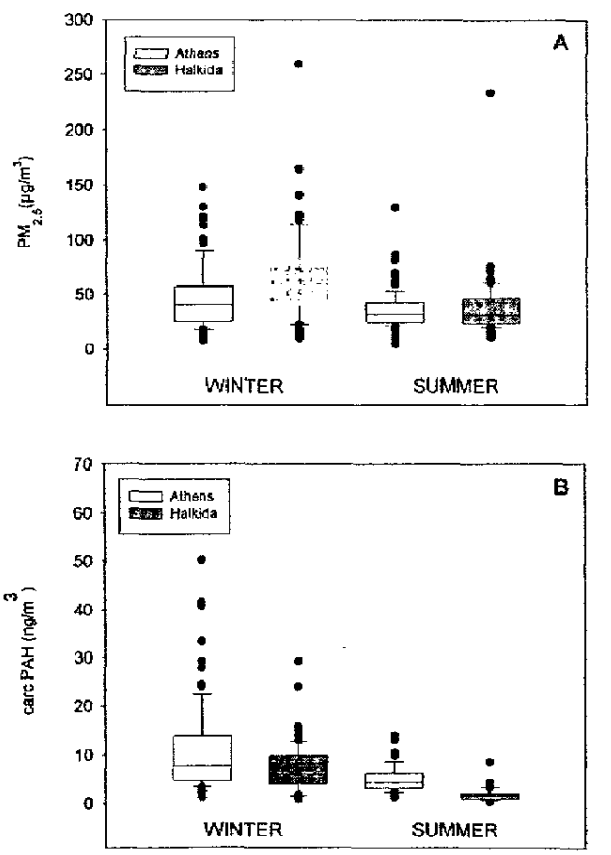

Figure 2. Personal $PM_{2.5}$ and total PAH exposures by location and season. (A) $PM_{2.5}$; (B) PAH. The horizontal lines in the boxes indicate the median value. Box boundaries indicate the 25th and 75th percentiles; capped bars indicate the 10th and 90th percentiles.

Table 2. Multiple linear regression analysis of the effects on personal PAH and $PM_{2.5}$ exposure of place of residence and declared time of ETS exposure during the last 24 h for Athens subjects.

	Total PAH					
	Winter (r=0.31)			Summer (r=0.23)		
	β coefficients	SE	P	β coefficients	SE	P
Address (1) Greater Athens Area/(2) centre of Athens	0.256	0.143	0.076	0.193	0.102	0.062
Declared time of ETS exposure during the last 24 h	0.176	0.062	0.005	−0.07	0.048	0.159
	$PM_{2.5}$					
	Winter (r=0.41)			Summer (r=0.12)		
	β coefficients	SE	P	β coefficients	SE	P
Address (1) Greater Athens Area/(2) centre of Athens	0.256	0.117	0.031	0.087	0.095	0.366
Declared time of ETS exposure during the last 24 h	0.227	0.053	<0.001	0.044	0.046	0.343

Greater Athens Area refers to the residential area 450 km^2 of Athens excluding the centre of Athens (Municipality of Athens).

P<0.05. Detailed statistical analysis did not reveal any correlation between personal exposures to $PM_{2.5}$ or PAH and cooking (e.g., frying or broiling), type of home heating, and use of different means of transport. A detailed analysis of the parameters that affect personal exposure to $PM_{2.5}$ and PAH will be presented elsewhere.

PAH Exposure Profile

Examination of the absolute amounts of individual PAH to which subjects in Athens and Halkida were exposed shows similarities as well as characteristic differences in the PAH exposure profiles. At both locations and during both seasons, the concentrations of the lighter PAH (including B[a]A, CHRYS, B[k]F, and B[b]F) were relatively low, while those of the heavier PAH, BPer and IND, were relatively high (Figure 3A). On the other hand, while the geometric means of the BPer and IND levels in Athens during winter exceeded substantially and significantly those in Halkida (by 99%, P<0.001 and by 54%, P=0.001, respectively), the absolute levels of CHRYS were almost significantly higher in Halkida (by 28%, P=0.06). As the concentration of B[a]P relative to the total PAH was similar for the two locations and at both seasons (13.9% and 14.1% during winter and 9.7% and 9.2% during summer for Athens and Halkida, respectively), the ratio of individual PAH to B[a]P can be used to reveal in a clearer way the differences in the PAH profiles at the two locations. As can be seen in Figure 3B, the ratios B[a]A, CHRYS, B[k]F, and B[b]F relative to B[a]P were higher in Halkida during both seasons (P<0.001), while those of BPer and IND were higher in Athens (P<0.001 and P=0.012 for BPer and P=0.073 and P=0.048 for IND for winter and summer, respectively).

Relationships between PAH and $PM_{2.5}$ Exposures

Exposures to individual PAH were highly correlated to $PM_{2.5}$ exposures. However, differences in the correlation

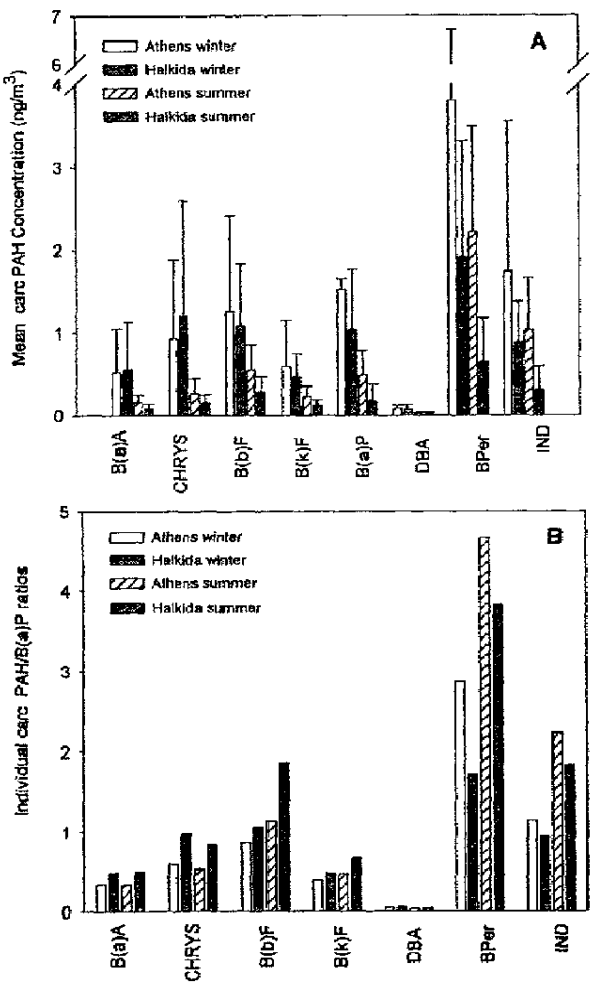

Figure 3. (A) PAH profile (mean values) of exposure to individual PAH in Athens and Halkida subjects by season. Error bars: SD. (B) Mean individual PAH/B[a]P ratios by location and season.

Table 3. Pearson correlation coefficients for the association of $PM_{2.5}$ with individual PAH and total carcinogenic PAH concentration.

PAH*	B[a]A	CHRYS	B[b]F	B[k]F	B[a]P	BPer	IND	Total PAH
Athens								
Winter	0.81	0.81	0.70	0.70	0.66	0.54	0.56	0.66
Summer	0.46	0.46	0.68	0.69	0.52	0.57	0.58	0.56
Halkida								
Winter	0.85	0.87	0.78	0.73	0.71	0.54	0.55	0.78
Summer	0.37	0.43	0.68	0.60	0.49	0.49	0.48	0.57

All correlations were highly significant ($P<0.001$).
*Most of the samples showed levels of DBA around the limit of detection; for this reason, the data for this PAH were used only for the estimation of the concentration of total carcinogenic PAH.

coefficients existed depending on the particular PAH, location, and season (Table 3). For all PAHs, winter correlation coefficients were higher in Halkida than the respective coefficients in Athens — B[a]A and CHRYS showing the best, and BPer the poorest, correlations with $PM_{2.5}$, regardless of location. Summer samples collected at both locations showed similar correlation coefficients to those of the winter samples for B[b]F, B[k]F, BPer, and IND, while the coefficients for B[a]A, CHRYS, and B[a]P were significantly reduced.

ETS, Cotinine, and PAH Exposure

Tobacco smoke exposure would be expected to make a significant contribution to personal PAH exposure as well as genotoxic burden. For this reason, as already indicated, all study subjects were non-smokers by declaration. However, ETS could still be an important source of exposure to airborne PAH and other genotoxins. After exclusion of nine subjects with cotinine levels above 20 ng/ml in one of their plasma samples (Figure 1), Halkida samples had higher cotinine levels than Athens samples during both seasons ($P<0.001$) (Table 4). In addition, at both locations, winter levels of cotinine were higher than the respective summer values ($P<0.001$).

An estimate of the time of exposure to ETS during the 24 h of exposure monitoring was obtained using data from the TLAD. Out of the 388 TLADs obtained from 194 subjects during winter and summer, 150 declared no ETS exposure, while 233 indicated ETS exposures ranging from 25 min to 10 h (five subjects did not provide information on their ETS exposure). During winter, subjects from Athens declared that they were exposed to ETS for a shorter period than Halkida subjects ($P<0.001$), while during summer, the difference was not significant ($P=0.190$) (Table 4). Both Athens and Halkida subjects declared longer exposures during winter than during summer ($P=0.022$ and $P<0.001$, respectively).

Sidestream smoke, the primary contributor to ETS, is known to be particularly rich in CHRYS and poor in BPer (IARC, 1979; Grimmer et al., 1987; Salomaa et al., 1988) as compared to diesel and gasoline combustion mixtures (IARC Monographs, 1983; Westerholm and Egeback, 1994). Although wood and coal combustion also results in CHRYS-rich PAH mixtures (Cretney et al., 1985; Daisey et al., 1986), these fuels are rarely used in Greece and the questionnaire data indicated that they were not used for residential heating of the study subjects. Consequently, it seems possible that the CHRYS/BPer ratio may act as a useful marker of ETS exposure of the study subjects. This suggestion is supported by the data in Table 5 which shows the geometric means of the cotinine concentrations and the CHRYS/BPer ratios for subjects declaring no or some ETS exposure at the two locations and the two monitoring seasons. It can be seen that cotinine levels and the CHRYS/BPer ratios were statistically significantly higher in the group declaring some ETS exposure, regardless of location or season. It can also be seen that both cotinine concentrations and the CHRYS/BPer ratios for the group declaring some ETS exposure were higher in Halkida than in Athens, in agreement with the more extensive ETS exposure declared in the respective TLAD. However, it is notable that a similar difference was also observed in the group declaring no ETS exposure (Halkida>Athens, $P=0.001$ and $P<0.001$ for plasma cotinine and the CHRYS/BPer ratios, respectively). This suggests that the TLAD may underreport the true extent of exposure to ETS, particularly in Halkida.

Table 6 shows the correlation between declared time of ETS exposure, plasma cotinine, and the CHRYS/BPer ratio. It can be seen that statistically significant correlations among all three markers were observed for the location-

Table 4. Cotinine level in plasma and declared ETS exposure during the last 24 h at both locations and during both seasons of the study; *P* values were obtained for cotinine from Students' *t*-test of the ln-transformed values, while for ETS exposure, from the non-parametric Mann–Whitney *U*-test.

	Mean	Median	Geometric mean	Range	*P*
Cotinine (ng/ml)					
Winter, Athens	2.39	1.60	1.72	0.30–11.60	<0.001
Winter, Halkida	5.14	4.09	3.92	0.36–16.70	
Summer, Athens	1.59	0.90	1.03	0.18–12.05	<0.001
Summer, Halkida	3.06	1.92	2.00	0.16–18.94	
ETS (hours of exposure during the last 24 h)					
Winter, Athens	0.83	0.50	–	0–6.75	<0.001
Winter, Halkida	2.28	1.75	–	0–8.75	
Summer, Athens	0.59	0.00	–	0–6.25	0.190
Summer, Halkida	1.55	0.00	–	0–10	

Table 5. Comparison of cotinine levels and CHRYS/BPer ratios for subjects declaring no ETS *versus* some ETS exposure.

Variables	ETS exposure (h)	Both seasons			Winter			Summer		
		N	Geometric mean	*P*	*N*	Geometric mean	*P*	*N*	Geometric mean	*P*
Both locations										
CHRYS/BPer	0	137	0.15	<0.001	49	0.20	<0.001	88	0.13	0.006
	>0	216	0.27		133	0.36		83	0.17	
Cotinine	0	150	1.29	<0.001	51	1.66	0.001	99	1.13	0.017
	>0	230	2.22		143	2.72		87	1.58	
Athens										
CHRYS/BPer	0	91	0.12	<0.001	38	0.16	0.002	53	0.10	0.032
	>0	120	0.18		69	0.24		50	0.13	
Cotinine	0	98	1.07	0.001	40	1.43	0.063	58	0.88	0.041
	>0	132	1.59		77	1.90		55	1.23	
Halkida										
CHRYS/BPer	0	46	0.22	<0.001	11	0.42	0.199	35	0.18	0.025
	>0	96	0.45		64	0.56		32	0.28	
Cotinine	0	52	1.82	<0.001	11	2.87	0.165	41	1.61	0.073
	>0	98	3.47		66	4.13		32	2.44	

pooled samples, particularly in the winter period. When samples were classified according to location and season of observation, the correlation became poorer and less consistent, with the exception of the correlation between ETS and CHRYS/BPer ratio, which remained significant during winter at both locations.

Exposures of Subjects Living in the Halkida TEI Campus Area

Of the 77 study subjects attending Halkida TEI, 40 lived in the village of Psahna where the institute campus is located, including 35 who lived at the TEI students' dormitory on the campus. Psahna village is located approximately 12 km from the town of Halkida, in rural surroundings characterised by a low traffic burden and the absence of any industrial activity. Examination of the exposure parameters of this subgroup of subjects (which will be referred to as the "Halkida campus area group") reveals that their $PM_{2.5}$ and PAH exposure levels were similar to those of the remaining Halkida subjects, almost all of whom lived in more urban areas (i.e., the town of Halkida or its suburbs) (Figure 4). However, there were differences in the ratios of individual PAH to B[*a*]P, with the Halkida campus area subgroup showing the highest ratios for the lighter PAH (e.g., B[*a*]A and CHRYS) and the lowest ratios for the heavy PAH (e.g., BPer and IND) regardless of season (Figure 4).

Similarly, plasma cotinine levels (Figure 5), declared ETS exposures (Figure 6), and the CHRYS/BPer ratios (Figure 7) of the Halkida campus area were higher than those found for the rest of Halkida subjects, for whom, in turn, these parameters were higher than those of Athens subjects. These differences were consistently observed during all four monitoring seasons, and their trend to vary in the order Athens<remaining Halkida<Halkida campus area was statistically significant ($P<0.05$).

Table 6. Linear regression analysis of (a) cotinine levels and hours of declared ETS exposure or CHRYS/BPer ratio and (b) declared ETS exposure and CHRYS/BPer ratio.

Variables	Both locations		Athens		Halkida	
	R	*P*	*R*	*P*	*R*	*P*
A	Cotinine					
ETS, winter	0.343	<0.001	0.150	0.105	0.258	0.023
ETS, summer	0.240	0.001	0.216	0.022	0.160	0.176
CHRYS/BPer, winter	0.322	<0.001	0.073	0.459	0.126	0.280
CHRYS/BPer, summer	0.185	0.016	0.036	0.723	0.092	0.463
B	ETS					
CHRYS/BPer, winter	0.454	<0.001	0.384	<0.001	0.254	0.028
CHRYS/BPer, summer	0.249	0.001	0.089	0.369	0.177	0.153

Discussion

The results reported here were obtained in the context of a molecular epidemiology study to investigate biomarkers of genotoxicity of urban air pollution. In this paper, we discuss the exposures of the study cohorts to $PM_{2.5}$ and PAH and their relationship with some possible determinants, especially exposure to ETS, while correlations with biomarkers will be presented in subsequent publications.

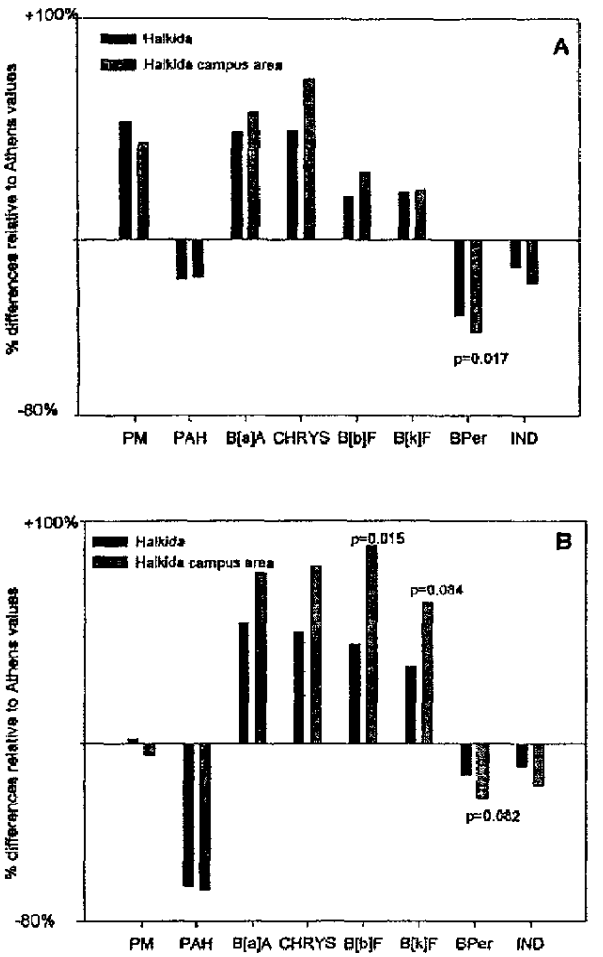

Figure 4. Comparison of $PM_{2.5}$ or PAH exposure levels and individual PAH/B[*a*]P ratios in Halkida subjects living in Halkida or in the Halkida campus area relative to Athens subjects. The bars represent the mean percent differences (positive or negative) of the ln values from the respective values observed in Athens. Statistical differences < 0.1 between personal exposure of Halkida and Halkida campus area residents are indicated. (A) Winter and (B) Summer.

Measurement of plasma cotinine levels confirmed the non-smoking status of the subjects during the 24-h exposure monitoring period (Figure 1). One hundred seventeen of the subjects studied lived in Athens and attended the Athens TEI, which is located in a densely populated part of the city with a heavy traffic burden. Based on questionnaire information, most of the Athens subjects lived within the greater urban complex of the city and commuted to the college by various private or public means of transport. The second cohort of 77 subjects investigated consisted of students attending the Halkida TEI. While no information on ambient air pollution in Halkida is available, based on the size and low population

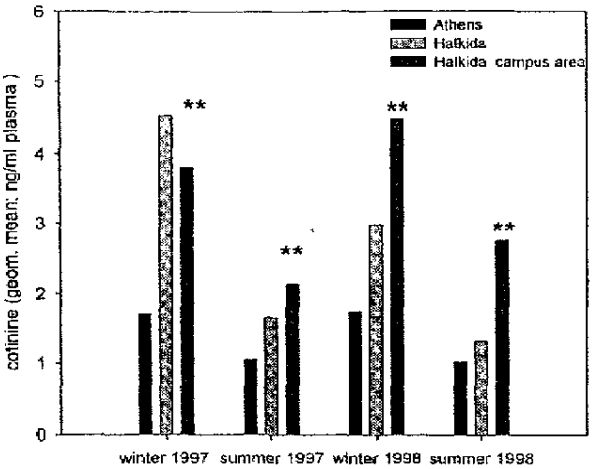

Figure 5. Comparison of plasma cotinine levels in subjects living in Athens, in Halkida, and in the Halkida campus area. **Significance for linear trend, $P < 0.001$.

density of this town as well as the limited industrial activity within a radius of 10 km, it is expected that ambient air pollution levels are likely to be lower than those of Athens. As in the case of Athens, traffic (consisting primarily of private, gasoline-driven cars, and diesel-driven buses and trucks) is expected to constitute the main source of ambient air PAH, with diesel-fired central heating also making a significant contribution in the winter. Based on questionnaire data, the location of residence of Halkida subjects included the town itself and its suburbs, while a significant number (35) lived in the students' dormitory

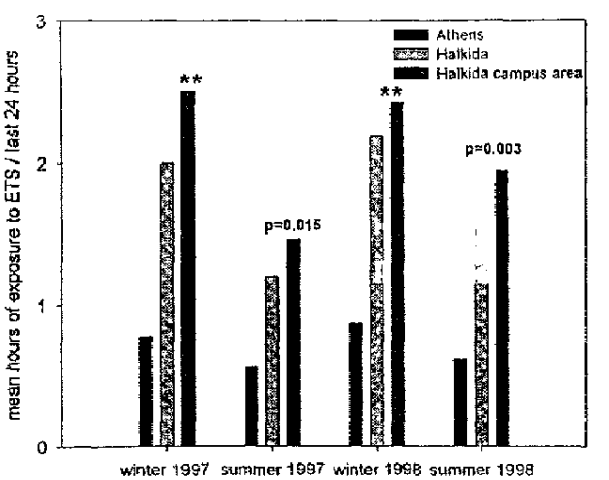

Figure 6. Comparison of declared ETS exposures in subjects living in Athens, in Halkida, and in the Halkida campus area. **Significance for linear trend, $P < 0.001$.

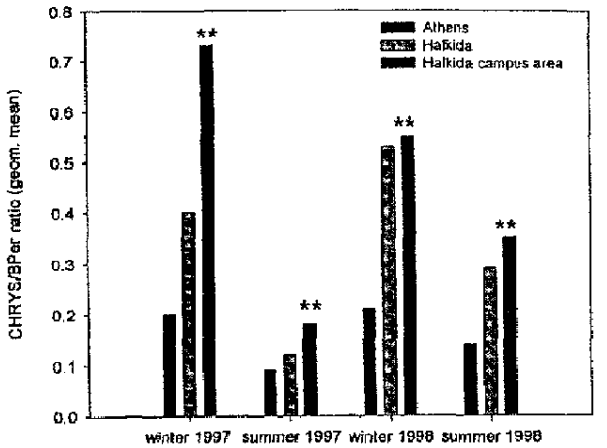

Figure 7. Comparison of CHRYS/BPer ratios in subjects living in Athens, in Halkida, and in the Halkida campus area. **Significance for linear trend, $P < 0.001$.

within the TEI campus, which is located in rural surroundings. The campus (including the dormitory) is of modern construction, with central heating. Students living outside the campus reported that they commuted to the institute by private or public transport, travelling a distance of approximately 12 km from Halkida through a mostly rural region.

The geometric means of $PM_{2.5}$ personal exposures observed in Athens during the winter and summer periods were 39.7 and 32.3 $\mu g/m^3$ (Table 1), respectively. These exposures are similar to those reported for non-smoking subjects who, for occupational reasons, spent much time outdoors in the relatively polluted region of Teplice in the Czech Republic (Binkova et al., 1996). Surprisingly, the corresponding levels for Halkida subjects were similar to, or, for the winter period, higher that those found in Athens (56.2 and 32.3 $\mu g/m^3$, winter and summer, respectively). This unexpected finding may reflect the operation of unknown sources of ambient particulate pollution in Halkida, or it may indicate differences in the personal behavior patterns of the subjects at the two locations, such as times spent indoors and outdoors, exposure to tobacco smoke, *etc.* Further analysis of the exposure determinants using questionnaire data may provide further insights into the origin of this high exposure.

The geometric mean levels of personal exposure to particulate-bound PAH in Athens (8.26 ng/m^3 in the winter and 4.44 ng/m^3 in the summer) (Table 1) were lower than anticipated, based on ambient concentrations of PAH reported for 1984 (Viras et al., 1987). Ambient air PAH concentrations at four different stationary stations in Athens were reported in that study to be higher than the personal exposure values found in the present study. This difference may reflect the improvement in the air pollution situation in Athens which has taken place during the past few years. The corresponding levels for Halkida subjects were significantly lower than those of Athens during both monitoring seasons (1.5-fold and 2.7-fold for winter and summer, respectively) (Figure 2). In a recently reported study, ambient atmospheric PAH levels were measured in six cities in Northern Greece, including some towns which could be considered comparable to Halkida in terms of population density, economic activity, and location. During the winter period, the levels found were comparable to the personal levels of Athens and Halkida subjects found in the current study, while for the summer period, the levels observed were significantly lower than those observed at both locations in the present study (Papageorgopoulou et al., 1999).

The fact — that while $PM_{2.5}$ exposures in Halkida were similar to or even higher than those observed in Athens subjects, the corresponding PAH exposures were significantly lower, especially in the summer (Figure 2A and B) — suggests the operation of different sources, with different PAH compositions of the corresponding $PM_{2.5}$, at the two locations. The diversity of sources and $PM_{2.5}$ composition is also reflected in the variable strength of correlation between individual PAH and $PM_{2.5}$ (Table 3), reflecting the variation of PAH composition of particulates emitted by different sources contributing to each subject's overall exposure. For example, BPer, which is a major component of the PAH associated with particulates produced by gasoline-driven motor vehicles (Westerholm and Egeback, 1994) and only a minor component in particulates originating from sidestream tobacco smoke (Grimmer et al., 1987), showed the weakest association regardless of season and location. An additional factor, which probably contributes to variation in the correlation coefficients between $PM_{2.5}$ and PAH, especially the reduction observed during the summer for some PAHs (B[*a*]A, CHRYS, and B[*a*]P), may be the photoreactivity or the gaseous particulate distribution of these PAHs (see further discussion below). The observed variability in the strength of the association of different PAHs with $PM_{2.5}$ exposure levels suggests that the latter cannot, by themselves, be employed as quantitative indicators of specific PAH in the context of molecular epidemiology studies where specific biomarkers might be related to specific PAH.

Operation of diverse particle sources can also explain why similar $PM_{2.5}$ values, but 3-fold higher PAH values, were observed in Teplice as compared to Athens (Binkova et al., 1996). For example, it is likely that the intensive use of coal for residential heating in Teplice, as compared to the preponderance of diesel-powered central heating in Athens, may significantly contribute to the high PAH exposures seen at the former location in winter. The mild winter and hot summer climate usually observed in Greece

relative to the cold climate of Teplice could also contribute to the observed differences due to the temperature-dependent distribution between the particle and gaseous phase of the more volatile PAH (see below in the discussion; Bodzek et al., 1993).

As already mentioned, different sources of PAH are characterised by particular PAH profiles, and a qualitative indication of the contribution of individual sources may be obtained by examination of the PAH profile observed in each case (based on such differences, attempts are being made to identify the contribution of different sources to the ambient aerosol mass concentration (Pistakopoulos et al., 1990). For example, because the BPer content of gasoline *exhaust* gases is relatively high, it has been suggested that the BPer/B[*a*]P ratio may reflect the contribution of gasoline-driven vehicles to atmospheric PAH pollution (Daisey et al., 1986). This ratio has been reported to have values above 2.5 in traffic tunnels (Handa et al., 1980; Kebbekus et al., 1983) and in areas with heavy traffic (Greenberg et al., 1985; Valerio et al., 1992; Brown et al., 1996; Nielsen et al., 1996), while a ratio of 4.0 has been reported for automobile exhaust fumes (Grimmer, 1977). The BPer/B[*a*]P ratio was found to vary significantly between the centre of Copenhagen, suburban, and rural areas, with the centre of the city exhibiting the highest, and rural areas the lowest values (Nielsen et al., 1996). The BPer/B[*a*]P ratios observed in the present study (2.9 for Athens and 1.7 for Halkida during the winter period) are compatible with traffic, particularly by gasoline-fueled cars, making an important contribution to personal PAH exposure in Athens and a less important contribution in Halkida. To our knowledge, only in one very recent study was the PAH personal exposure profile associated with possible outdoor sources of PAH exposure (Lewtas et al., 2000). In this study, the abundance of BPer (relative to B[*a*]A) was also found to be associated to the exposure to gasoline engine exhaust.

In contrast to the decreased BPer/B[*a*]P ratio found in Halkida, the CHRYS/B[*a*]P ratio, as well as the corresponding B[*a*]A, B[*k*]F, and B[*b*]F ratios, was higher in Halkida (Figure 3B), suggesting a greater contribution at this location of a source relatively rich in the lighter hydrocarbons. In an Italian study, the industrialised areas of two cities (Genoa and La Spezia) were reported to show significantly higher concentrations of ambient atmospheric B[*a*]A and CHRYS relative to the corresponding urban residential areas, suggesting that the preponderance of these light PAH may reflect the contribution of industrial pollution (Valerio et al., 1992). However, no major industrial activity exists in the immediate vicinity of Halkida. On the other hand, as already mentioned, side-stream tobacco smoke is rich in light PAH, including B[*a*]A and CHRYS, and could also be an important source of PAH exposure (see below).

Seasonal Variation

$PM_{2.5}$ and PAH exposures were significantly reduced during summer at both locations as compared to winter. While mean PAH exposure levels in Athens were reduced during the summer season by about 1.9-fold as compared to winter, the corresponding $PM_{2.5}$ exposures were reduced only 1.3-fold. In Halkida, the PAH and $PM_{2.5}$ exposure levels were reduced to an even greater degree during summer (3.9-fold and 1.75-fold, respectively) (Figure 2, Table 1). These variations may reflect seasonal changes in ambient concentrations as well as personal behaviour patterns. Seasonal variations in ambient PAH levels similar to those observed for personal exposures in the present study have been reported in a number of studies in European regions, with 2- to 5-fold lower levels being generally found during the summer (reviewed in Menichini, 1992). Large seasonal differences in ambient PAH concentrations were observed in a number of cities of northern Greece, with the winter-to-summer ratio ranging from 4 in the centre of Thessaloniki (with a population of over 1 million) to 20 in the small town of Kilkis; Papageorgopoulou et al., 1999). Domestic heating during winter, meteorological conditions (particularly temperature inversion during winter), temporary closure of factories during the summer, and increased chemical reactivity or photolytic decomposition of PAH during the summer period are some of the explanations given for the observed seasonal variation (reviewed in Nikolaou et al., 1984). Bodzek et al. (1993) observed higher winter-to-summer ratios for the lighter particle-associated PAHs such as B[*a*]A and CHRYS and lower ratios for the heavier PAHs. These authors attributed the PAH-specific differences to changes in the temperature-dependent distribution of the more volatile PAH between the particle and gaseous phase. In our study, while the winter-to-summer ratios, e.g., for Athens and for the B[*a*]A and CHRYS, are approximately 3, the corresponding ratios for B[*b*]F and BPer are 1.36 and 1.5, respectively (Figure 3A).

During summer, the BPer/B[*a*]P ratios increased at both locations and approached values of 4.7 for Athens and 3.8 for Halkida (Figure 3B). A seasonal increase in this ratio has been also reported elsewhere (Greenberg et al., 1985), while similar summer values were observed for ambient levels in cities of northern Greece (Papageorgopoulou et al., 1999), and even higher values have been reported for Stockholm (Colomsjo et al., 1986) and Toronto (Katz et al., 1978). The increase of BPer/B[*a*]P ratios (which are believed to reflect the contribution of gasoline exhaust) during the summer could be attributed to the contribution during winter to the PAH emission profile of diesel-powered central heating (Moller and Afheim, 1980).

An additional factor contributing to the increase of the BPer/B[*a*]P ratios in the summer may be the relatively high photolability of B[*a*]P and reactivity (as compared to

that of BPer) which leads to its selective degradation during the summer (IARC Monographs, 1983; Nielsen, 1984; Greenberg, 1989). Although PAH production may be the major factor determining the seasonal variation of ambient PAH levels in some cities (e.g., Paris; Pistakopoulos et al., 1990), it seems likely that in the present study, PAH decay and photoreactivity also play an important role. Summer in Greece is characterised by high temperatures (sometimes above 40°C), intense solar radiation, and (at least for Athens) high ozone concentrations, all of which can significantly reduce the lifetime of airborne PAH. Increased decay of B[*a*]P and B[*a*]A due to their high photosensitivity and reactivity, and altered distribution of the more volatile B[*a*]A and CHRYS between the particulate and gaseous phases probably account for the fact that, while the correlation coefficients of these PAHs to $PM_{2.5}$ levels observed in our studies were among the highest during the winter, they were among the lowest during the summer (Table 3). This suggestion is further supported by the fact that the proportions relative to the total PAH of only these three PAHs showed a strong, inverse correlation with the average temperatures during the days of monitoring (data only available for Athens, not shown).

Exposure to ETS and Its Influence on PAH Exposure Profiles

Apart from ambient air pollution, factors related to the personal microenvironment and reflecting personal behaviour patterns and lifestyles may also significantly modify the quantitative and qualitative exposure to airborne PAH of different subjects. Among such possible factors are exposure to ETS or to fumes encountered in the cooking process (e.g., frying or broiling), type of home heating, use of different means of transport, or other activities. Detailed information on subject's exposure *via* such activities was obtained through a 24-h recall questionnaire as well as the TLAD. Detailed statistical analysis did not reveal any correlation between personal exposures to $PM_{2.5}$ or PAH and any of the abovementioned activities, with the exception of exposure to ETS. In a study conducted in the Teplice district, no correlation was found between particle-associated PAH and nicotine levels (personal monitoring was conducted) and it was suggested that passive smoking did not contribute significantly to the existing ambient exposure in Teplice (Watts et al., 1994). While this could be the case in high ambient levels of PAH such as those found in Teplice, we do find a correlation between ETS and not the absolute PAH concentration but the relative CHRYS to BPer concentration (CHRYS/BPer ratio). Subjects declaring no ETS exposure in the 24-h period, during which personal monitoring was conducted, had a substantial (approximately 2-fold) and highly significant reduction in their CHRYS/BPer ratios as compared to subjects declaring some ETS exposure (Table 5). This finding was true regardless of location or season, with the only exception of Halkida subjects during the winter period, when, while the same trend was observed, the difference did not reach statistical significance. The half-life of cotinine elimination from plasma is about 24 h (Haley et al., 1989) and plasma cotinine levels obtained at the end of the 24-h personal monitoring period should reflect the ETS exposure during that period. In general, cotinine levels were also significantly lower for subjects declaring no ETS exposure, although the *P* values were lower than those observed for the dependence of the CHRYS/BPer ratios on the ETS exposure status (Table 5). This may be attributed to inter-individual differences in nicotine metabolism. Among subjects declaring no ETS exposure, those in Halkida had approximately 2-fold higher cotinine levels and CHRYS/BPer ratios as compared to the corresponding Athens subjects, suggesting that ETS exposure was consistently underreported by Halkida subjects.

In an attempt to investigate the quantitative relationship between the time of ETS exposure as declared in the TLAD, plasma cotinine levels, and CHRYS/BPer ratios, linear regression analyses were performed. For the pooled cohort regardless of location, statistically significant correlations were obtained for any two of these markers during both seasons (Table 6). When correlations at individual locations were examined, CHRYS/BPer ratios were found to be highly correlated with declared ETS exposure at both locations only during the winter period — this seasonal specificity probably reflecting more frequent indoor ETS exposure during winter. On the other hand, location- or season-specific cotinine levels exhibited no correlation with CHRYS/BPer ratios, while their correlation with declared ETS exposure was not consistent. However, there is still no general agreement on the degree to which cotinine can serve as a quantitative or semi-quantitative indicator of ETS exposure particularly at low levels of exposure (Henderson et al., 1989; Coultas et al., 1990a,b; Benowitz, 1999; Jenkins and Counts, 1999). In particular, inter-individual differences in the nicotine metabolism may have contributed to the poor quantitative correlation of plasma cotinine with the other ETS-related parameters observed in the present study. It is therefore concluded that the CHRYS/BPer ratio derived from the profile of personal PAH exposure reflects individual exposure to ETS of subjects in the present study and may be used to classify them for this variable. No significant or consistent correlation of the personal PAH exposure profile was found with any other relevant activities, as obtained from the questionnaire (such as traffic density at the place of residence) or from the TLAD (such as time spent indoors or outdoors or time spent on various cooking-related activities) (results not shown).

The relative abundance of lighter PAHs within the PAH exposure profile was higher, while that of heavy PAHs was lower, for Halkida subjects. Furthermore, the declared ETS

exposures, plasma cotinine levels, and CHRYS/BPer ratios of Halkida subjects clearly and consistently indicate that this group experienced significantly more exposure to ETS than Athens subjects. Within the group of Halkida subjects, a subgroup consisting of subjects living in the students' dormitory on the TEI campus showed the characteristic differences in the PAH exposure profiles (overrepresentation of light PAH and underrepresentation of heavy PAH) to an even greater degree than the remaining Halkida subjects (Figure 4). This subgroup, which had significantly increased plasma cotinine values and declared ETS exposure as compared to both other cohorts (Figures 5 and 6), also showed increased values of the CHRYS/BPer ratio in the PAH exposure profiles (Figure 7). The parallel variation of these three parameters among the three cohorts (lowest for Athens subjects, intermediate for Halkida subjects other than those living in Halkida campus area, and highest for subjects living in Halkida campus area) was consistently observed during both winter and both summer monitoring periods. Taking into account the rural location of the TEI campus, its low traffic burden, and the absence of any industrial activity in its vicinity, these observations further support the suggestion that the CHRYS/BPer ratio in the personal PAH exposure profile reflects ETS exposure.

Summarising the conclusions of this study by examining the 24-h personal exposures to airborne $PM_{2.5}$ and associated carcinogenic PAHs of 194 non-smoking subjects, we have found that (a) exposure to ETS can make a significant contribution to the overall personal exposure to PAH in subjects suffering from moderate to low exposures to urban air pollution; and (b) increased exposure to ETS of specific subcohorts is consistently reflected in higher relative exposure to light PAH and higher ratios of CHRYS/BPer in their PAH exposure profile. These findings may be utilized in the assessment of the determinants of biomarkers of genotoxicity measured in the same subjects.

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